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U. S. DEPARTMENT OF AGRICULTURE,

BUREAU OF ANIMAL INDUSTRY.—BULLETIN 111.

A. D. MELVIN, CHIEF OF BUREAU.

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CHEMICAL AND PHYSICAL STUDY OF THE LARGE AND SMALL FAT GLOBULES IN COWS' MILK.

BY

R. H. SHAW,
Assistant Dairyman, Dairy Division,

AND

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LETTER OF TRANSMITTAL.

U. S. Department of Agriculture,
Bureau of Animal Industry,
Washington, D. C., October 20, 1908.

Sir: I have the honor to transmit herewith, and to recommend for publication in the bulletin series of this Bureau, the accompanying manuscript of an article entitled "A Chemical and Physical Study of the Large and Small Fat Globules in Cows' Milk," by R. H. Shaw, of the Dairy Division of this Bureau, and C. H. Eckles, of the Missouri Agricultural Experiment Station. This work is a preliminary part of a comprehensive investigation being conducted at the Missouri station by cooperation between that station and the Dairy Division concerning the effects of period of lactation, feed, and other factors on the chemical composition of milk.

At the request of the authors acknowledgment is made to Messrs. J. O. Halverson and G. C. Payne for assistance in the chemical and microscopical work.

Respectfully,

A. D. Melvin,
Chief of Bureau.

Hon. James Wilson,
Secretary of Agriculture.

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A CHEMICAL AND PHYSICAL STUDY OF THE LARGE AND SMALL FAT GLOBULES IN COWS' MILK.

INTRODUCTION.

In planning a long investigation on the effect of the period of lactation, feed, and other factors on the chemical composition of milk it was decided that the most practical way of extracting the fat from the milk was by passing it through a small separator and churning the cream. In this method the tendency is for the smaller globules to escape in the skim milk and buttermilk; hence it became necessary to ascertain whether or not these small globules which in part escape differ in chemical composition from the large globules, and if such a difference exists, to determine just what it is.

Investigations on these points are not lacking, and had these investigations been sufficiently extensive, or had they agreed on the principal points, the work herein presented would have been unnecessary. However, such is not the case. There is almost a total lack of agreement in the work hitherto published, as will be seen later on. When the present investigation was taken up it was the intention to cover only those points necessary to validate certain determinations involved in the long investigation mentioned above. Later it was decided to enlarge it to some extent and publish it as a contribution to the knowledge of the chemistry of butterfat.

PREVIOUS INVESTIGATIONS.

Müller[a] (1867) observed that fat extracted from skimmed milk is waxlike, and also that in the analysis of butter where 100 grams or more of fat-free substance is obtained the last particles of fat extracted have more the properties of wax than of butterfat.

Schroeder[b] in 1872 published some investigations showing that when milk is fractionally creamed the butterfat obtained from the

[a] Müller, Alexander. Chemische untersuchungen auf dem gebiete der milchwirthschaft. Die Landwirthschaftlichen Versuchs-Stationen, Band 9, pp. 364-396. See p. 390. Chemnitz, 1867.

[b] Schroeder, G. Beiträge zur kenntniss des fettgehalts der milch. Milch Zeitung, No. 28, pp. 325-327, 1872.

first fraction has a lower melting point and lower specific gravity than the butterfat obtained from the last fraction. He also stated that the latter contained no butyric acid.

Klusemann[a] in 1893 conducted an investigation with milk from individual cows. He separated the large and small fat globules by passing the whole milk, at a temperature of 5 to 12° C., through a De Laval separator running at low speed. The cream contained the large globules. The skim milk was then warmed somewhat and again passed through the separator running more rapidly. The cream in this instance contained the medium-sized globules. The small globules were obtained by heating the last skim milk to 36 to 38° C. and again passing it through the separator, this time running very rapidly; the cream was churned in a small dash churn, and the resulting butter was melted and filtered by means of a jacketed filter.

He determined the melting and freezing points, the water-insoluble fatty acids, the melting and freezing points of the insoluble fatty acids, the iodin absorption number, the volatile acids, and the specific gravity. In his conclusions he states that the percentage of the insoluble fatty acids, and the melting point and freezing point of the butterfat, and of the insoluble fatty acids are higher in fat from small-globule cream than in fat from large-globule cream, the reverse being true of the percentage of volatile acids and of the iodin number.

Gutzeit[b] (1895) published an investigation on the same subject. He chose mixed milk from a herd of 150 cows. He separated the large and small globules as follows: He used 100 liters of milk, 20 liters of which was allowed to rise for six hours in a pan immersed in cold water. The resulting cream contained the large fat globules. The remaining 80 liters was then run through a separator, the cream discarded, and the skim milk coagulated. The whey was then run through a separator, and the resulting whey cream contained the small globules. The two creams were churned separately, and the resulting butters were heated for a time at 60° C. and filtered. He determined the specific gravity, the melting point, the refractive index, insoluble fatty acids, volatile fatty acids, saponification number, and iodin absorption number, and concludes that in homogeneous milk the fat globules of all sizes have the same chemical-physical composition.

---

[a] Klusemann, Erich. Die zusammensetzung und die beschaffenheit der aus den grossen und den kleinen fettkügelchen der kuhmilch gewonnenen butter. Inaugural dissertation. Leipzig, 1893.

[b] Gutzeit, Ernst. Die schwankungen der mittleren grösse der fettkügelchen in der kuhmilch nach laktation, fütterung und rasse, sowie über den physikalischen und chemischen unterschied der grössten und kleinsten fettkügelchen. Landwirthschaftliche Jahrbücher, Bans 24, pp. 539–668. Berlin, 1895.

Lemus[a] (1902) separated the large and small globules by interrupted milking. The first liter of milk drawn he took to represent the small globules and the last fraction of about one-half liter drawn the large globules. After reserving a sample for fat determination and microscopical examination, he extracted the fat according to the method of Soxhlet by shaking with ether after having first made it alkaline with potassium hydroxid. The ethereal layer was then removed and dried at 100° C. to a constant weight. He made some of the same determinations as did Klusemann and Gutzeit, but in his conclusions refers only to the volatile acids and the oleic acid. He states that in the majority of cases the smaller fat globules contained more oleic acid and less volatile acids than did the larger globules.

In the three more recent investigations, that is, those by Klusemann, Gutzeit, and Lemus, it is seen that no two draw the same conclusions. Gutzeit claims that there is no difference, chemically or physically, between the large and small fat globules in the same milk. Klusemann and Lemus agree in claiming that there is a difference, but disagree on some of the points of difference.

Klusemann worked on the milk of five individual cows and on the mixed milk of seven. A study of his results shows that in every case the percentage of insoluble acids in small-globule fat is higher than in the large-globule fat, the difference being from about 0.3 per cent to about 3 per cent; the melting point of the butterfat was higher in the small-globule fat than in the large, the variation being from 0.5 to 2° C.; the volatile fatty acids in terms of Reichert-Meissl number showed a decrease in the small-globule fat in six cases and an increase in two cases, the variation being from about 1 c. c. to about 4.5 c. c.; the iodin number in three cases showed practically no difference, in three cases it showed a decrease in the small-globule fat and in one an increase, the largest difference being about 2.5 per cent. Granting that no criticism can be made of his methods and work, it would seem that with the limited number of animals used his conclusions even on the insoluble fatty acids and melting point are unwarranted, and certainly not justified in the case of the other constants.

In Lemus's investigation it may be seen that the separation of the large and small globules is not positive. In fact, instead of the relative size of the fat globules being invariably larger in the stripper milk than in the foremilk, the reverse is true in some cases. In 10 cases the iodin number was smaller in the large-globule fat than in the small-globule fat, and in 3 cases it was larger, the difference ranging

[a] Lemus, Woldemar. Ueber die chemische beschaffenheit des in den grossen und in den kleinen milchkügelchen enthaltenen fettes. Inaugural dissertation. Leipzig, 1902.

from 0.11 to 3.54 per cent. With the volatile acids the Reichert-Meissl number was smaller in 16 cases in the large-globule fat than in the small-globule fat, and in 6 cases the reverse obtained, the differences ranging from 0.45 to 13.85 per cent. It is also doubtful whether the fat remained unchanged in the preparation of the samples, where a prolonged heating at 100° C. was necessary to eliminate the ether.[a] Moreover, it would seem that his determinations show the difference between the fat in the foremilk and that in the milk secreted during the process of milking, rather than that between the large and small fat globules in the same milk.

EXPERIMENTAL WORK.

For our investigation animals were selected which exhibited a wide range of breed, age, and period of lactation. In the earlier part of the investigation the chemical and microscopical work was limited to determinations of the relative size of the fat globules, the iodin number, the Reichert-Meissl number, the Koetstorfer number, and the refractive index. Later it was extended to include the specific gravity, the melting point, the Hehner number, the color, and the percentage of different-sized fat globules.

Table 1 gives the data concerning the animals. In Tables 2, 3, and 4 will be found the results of the chemical and microscopical work.

SOURCE OF THE SAMPLES.

The cows selected to supply the samples represented four breeds—Jersey, Holstein-Friesian, Ayrshire, and Shorthorn—and were members of the Missouri Agricultural College herd. Groups supplying the samples were arranged with a view of having certain samples represent milk from cows in the early part of the period of lactation, while others represented milk produced in the latter part of the period. The table shows the number of days in milk, the breed, the age, the date at which samples were taken, and the average percentage of fat in the milk of each cow. The sample of milk taken was the total product for one day of the group of cows shown in the table.

METHOD OF SEPARATION.

The first method tried consisted in allowing the milk to stand at room temperature for about two hours until a portion of the cream had come to the surface, when it was removed by drawing the remainder of the milk from a faucet in the bottom of the can. The partially

[a] According to C. A. Brown (Annual Report of the Pennsylvania State College, 1899–1900, p. 208), a temperature much above 50° C. will very soon alter the composition of butterfat. Butterfat kept at 50° C. for two days showed a loss of over one unit in iodin number.

skimmed milk secured in this way was again placed in the can and allowed to stand about twelve hours, when the second portion of the cream was removed as before. This skim milk, which still contained approximately 0.3 per cent of fat, was then run through a centrifugal separator at a temperature of 32° C. The cream thus secured contained the smallest fat globules which it is possible to obtain by the centrifugal machine. The cream secured from the first skimming contained the larger fat globules, as they reach the surface first under the influence of gravity, but it also contained some small fat globules. In order to eliminate these this cream was placed in the bottom of a deep, narrow can, which was then filled with the skim milk from the final separation with the centrifugal separator. When most of the cream had again risen it was skimmed off and retained for the sample of the large fat globules.

The above method was found to be satisfactory, but equally good results were secured, at a considerable saving of time, by using the centrifugal separator. After several preliminary trials the following plan was adopted:

The milk was first run through the centrifugal separator at a temperature of 30° to 32° C. and the speed of the machine so regulated by trial that approximately one-half of the fat in the original milk was taken out as cream and one-half remained in the skim milk. It was found that with a hand-power separator, the normal speed of which was 60 revolutions of the crank per minute, this result was obtained when the machine was operated at the rate of 15 to 25 revolutions a minute, depending upon the character of the milk separated. The cream secured from this separation contained the larger fat globules, but also a large number of medium sized and some small ones, which were removed as described later.

The skim milk secured by this separation contained approximately one-half the fat originally found in the milk, and was again separated at a higher speed—from 29 to 40 revolutions of the crank to the minute. The speed was adjusted after trial with each sample of milk so that about 0.2 per cent of fat remained in the skim milk. The cream from this second separation was rejected, as it contained medium sized and some large fat globules which had escaped the first separation.

The skim milk containing approximately 0.2 per cent of fat was then heated to 38° C. and again run through the centrifugal separator at a speed of from 65 to 70 revolutions of the crank to the minute. The cream from this separation contained the smallest fat globules which it is possible to secure with a centrifugal separator. It is impossible to obtain all of the fat by any mechanical method of separation. The fat remaining in the skim milk after this final separation was found to be from 0.025 to 0.004 per cent, as shown by the Babcock method, using the double-necked Wagner testing bottle.

The cream from the first separation containing the largest fat globules was added to the skim milk from the third and run through the separator again at the speed used in the first separation, to eliminate the smaller fat globules which had remained with the large. The cream from this separation was again mixed with a second lot of the same skim milk from the third separation and the process repeated, this cream being taken as the final sample containing the largest fat globules. The two lots of cream were then churned by shaking in a closed jar as long as any butter could be accumulated.

PREPARATION OF FAT SAMPLES FOR ANALYSIS.

The butter was immediately melted on a steam bath and allowed to remain in this condition until the curd and water had settled. Special care was taken at this point that the temperature did not rise above 50° or 55° C. and also that it remained, even at this temperature, no longer than was necessary. It was then filtered through a paper filter kept warm by an electrical device, and preserved in corked bottles which were protected from light in a refrigerator until the butter was analyzed.

ANALYTICAL METHODS.

The methods of the Association of Official Agricultural Chemists were employed whenever possible. Duplicate determinations were made in all cases and in some triplicate or even quadruplicate. Only the briefest description of the official methods used is given below. For a detailed description reference may be made to Bulletin 107 of the Bureau of Chemistry, United States Department of Agriculture.

Specific gravity.—The specific gravity was determined in small pyknometer bottles at the temperature of boiling water.

Melting point.—The melting point was determined according to Wiley's method, by placing a disk of the fat in a large test tube containing boiled distilled water and boiled alcohol which had been cooled, the tube being placed in a beaker of water which was slowly heated, and noting, by means of a thermometer graduated to 0.1° C., the temperature at which the disk assumed the form of a sphere.

Refractive index.—The refractive index was determined with a Zeiss-Abbe refractometer which had been standardized with distilled water. The fat was kept at a constant temperature above its melting point during the determination by means of a current of warm water circulating through the instrument. The reading was reduced later by means of a factor to 25° C.

Volatile acids.—The method of Reichert, modified by Meissl, was employed in the estimation of the volatile acids, and the results are given as Reichert-Meissl numbers. In saponifying, use was made of the Leffman-Beam method, in which the fat is saponified in a flask

over a naked flame with a mixture of a strong aqueous solution of caustic soda and pure glycerol. The soap obtained in this way is decomposed by sulphuric acid and the liberated volatile acids are distilled over with the steam and titrated with decinormal barium hydroxid solution.

Saponification value.—The saponification value, or Koettstorfer number, was determined by the regular official method. The fat is saponified in a flask on a steam bath with an alcoholic potash solution, using a long glass tube as reflux condenser.

Iodin absorption number.—The method of Hübl was employed in this case. The fat is dissolved in chloroform and subjected to the action of a mixture of an alcoholic solution of iodin and of mercuric chlorid in a dark place for three hours, at the end of which time the unabsorbed iodin is determined by titrating with standard sodium thiosuphate solution. The percentage of iodin absorbed by the fat is expressed in the results as the iodin number.

Insoluble fatty acids.—For this determination the official method, or that of Hehner, as it is called, was employed. It was found, however, that better results were obtained by increasing the amount of fat to 10 grams, as suggested by Brown.[a] The results are given as Hehner numbers.

COLOR DETERMINATIONS.

The color determinations were made with the Lovibond tintometer, using the standard color glasses. While this instrument is not admirably adapted to the work with milk and cream, so far as the authors' knowledge goes, it is the best that is available. With the melted fat where the light is transmitted through a considerable layer the determinations can be made quite accurately, but with the opaque milk, cream, and butter, where the light must be reflected from the surface, very slight differences in color can not be determined. The results are given in terms of the numbers assigned to the standard color glasses.

MICROSCOPICAL WORK.

In the microscopical part of the work the method devised by Dr. S. M. Babcock[b] was used. The milk is diluted with distilled water to fifty times and the cream to one hundred times its volume. Fine capillary tubes are drawn out from larger tubes, care being taken that the resulting tubes are round in cross section. They are then broken into pieces 2 to 3 centimeters in length, and by means of forceps each tube is filled by immersing one end in the diluted cream or milk. The tube fills instantly by capillary attraction.

[a] Report of Pennsylvania State College, 1899–1900, p. 211.

[b] Fourth Annual Report of the New York Agricultural Experiment Station, 1885.

The two ends are then sealed with vaseline. Three such tubes are used for each sample, and these are laid parallel on a slide. A drop of glycerin is then placed over the tubes and a cover glass applied. The slide thus prepared is allowed to remain on a leveled slab for half an hour, the purpose of this being to allow the globules to rise to the top of the capillary tubes. At the end of thirty minutes the slide is placed under a microscope provided with an ocular micrometer and a mechanical stage. The number of globules in 50 divisions of the micrometer scale are counted in three places in each tube, the internal diameter of the tube at each place of counting being first deter-

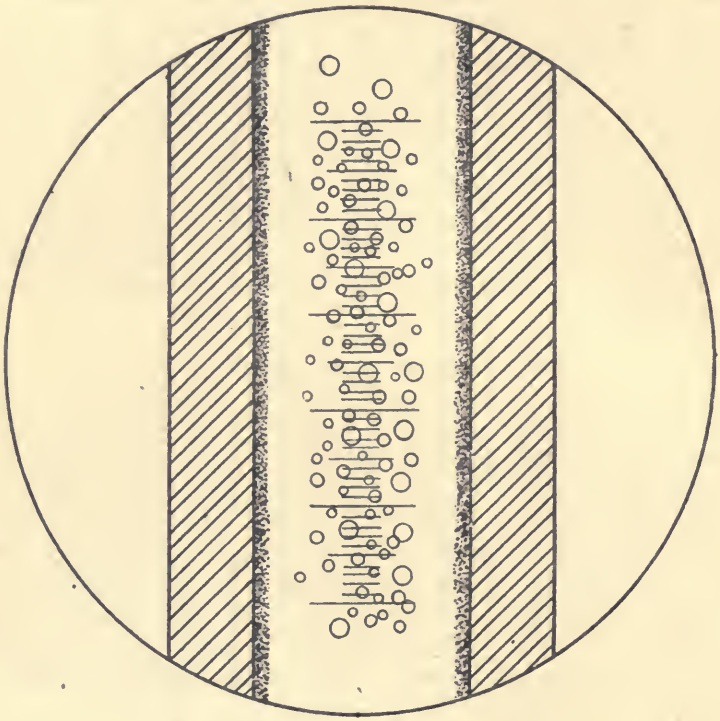

FIG. 1.—Showing method of counting globules.

mined by throwing the ocular micrometer scale across the tube at right angles and carefully counting the divisions within the two walls. The total number of globules in the 50 divisions is recorded, as well as the number under one division in diameter; also the number between one and two divisions in diameter, and the number having a diameter greater than two divisions. A 1-inch ocular and one-sixth inch objective are employed, and with this combination the value of each division of the ocular micrometer with the instrument used is 0.00258 millimeter. In this way nine determinations are made for each sample.

In order to compare results, the number of globules which would be found in a standard tube 100 divisions in diameter and 50 divisions in height is calculated from the number in each observed tube and the average taken. In making this calculation the formula $\frac{10{,}000\,n}{d^2}$ is used.[a] With the ocular micrometer used in this work the standard tube would have a volume of 0.006744 cubic millimeter, and if the milk is diluted to fifty times its volume, 0.006744 divided by 50, or 0.00013488 cubic millimeter, would be the volume of the original milk contained in the standard tube. In the case of cream where it is diluted to one hundred times its volume one-half this figure represents the volume of original cream in the standard tube. To find the number of globules present in 0.0001 cubic millimeter it is only necessary to

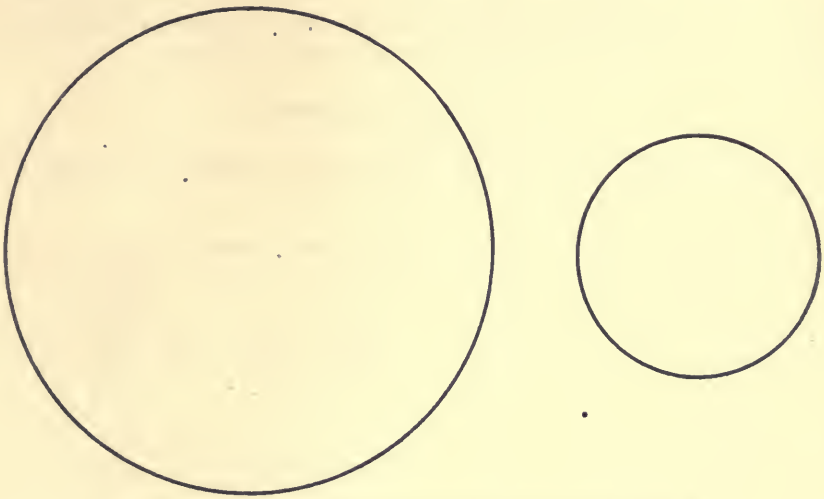

FIG. 2.—Showing the average relative size of large and small fat globules.

multiply the number in the standard tube by the factor 0.7414, obtained by dividing 0.0001 by 0.00013488.

The relative size is found by dividing the percentage of fat by the number of globules in 0.0001 cubic millimeter of the milk or cream. To avoid fractions, the figure obtained in this way is multiplied by 10,000.

An example illustrating the method of calculating may be given. A sample of milk containing 4 per cent of fat is chosen. Let 30 represent the number of globules counted in 50 divisions of a tube whose

[a] The volume of two cylinders having the same height but different diameters are to each other as the squares of their diameters. If we let n equal the number of globules in the observed tube, n'' the number in the standard tube, d the diameter of the observed tube, and 100 the diameter of the standard tube, then $n : n'' : : d : 10{,}000$, or n'' equals $\frac{10{,}000\,n}{d^2}$.

internal diameter has been found to be 40 divisions. Applying the formula $\frac{10{,}000\ n}{d^2}$, we have $\frac{300{,}000}{1{,}600}$ =187, or the number of globules which would be found in a standard tube whose length is 50 divisions and whose diameter is 100 divisions. Multiplying 187 by the factor 0.7414, we have 138, which would be the number of globules in 0.0001 cubic millimeter. Dividing 4 by 138 and multiplying by 10,000, we have 290 as the relative size of the fat globules in the example taken. As Doctor Babcock states, the relative size is not strictly accurate, since percentages by weight have been confused with volume. To be strictly accurate we must multiply the relative size by a factor obtained by dividing the specific gravity of the milk by the specific gravity of the butterfat. However, since relative values only are required in our work, it was decided that the relative size would answer the purpose, so our calculations have stopped at that point and the results are expressed in terms of relative size.

RESULTS OF EXPERIMENTS.

The following tables present data as to source of samples and results of the experiments:

TABLE 1.—*Data concerning source of samples.*

Sample No.	Date.	Breed of cow.	Date of last calving.	Time in milk.	Age of cow.	Daily yield of milk when sample was taken.	Average fat content.
	1906		1906.	*Days.*	*Yrs. Mos.*	*Pounds.*	*Per cent.*
		Ayrshire	July 10	77	2 5	21.5	4.0
1	Sept. 25	....do	Aug. 13	43	3 8	28.9	3.9
		....do	July 29	58	3 2	25.1	4.0
		Jersey	May 8	151	9 2	27.9	4.5
2	Oct. 3	....do	Aug. 19	45	9 1	30.3	4.1
		....do	May 8	148	9 0	22.1	5.0
		....do	Dec. 15	298	8 11	10.3	5.6
		....do	Jan. 26	256	2 7	11.1	5.2
3	Oct. 9	....do	Feb. 7	244	3 3	10.3	5.8
		....do	Dec. 6	307	8 11	7.4	6.7
		....do	Jan. 25	257	4 7	7.5	5.5
		....do	Feb. 6	345	9 0	9.1	5.0
		Holstein	Apr. 18	193	6 4	32.2	3.2
4	Oct. 28	....do	Apr. 29	182	4 6	20.6	3.0
		....do	May 13	168	4 5	31.9	3.4
		....do	Feb. 11	259	4 4	23.1	3.0
	1908.		1907.				
5	Jan. 17	Shorthorn	Sept. 30	109	4 8	19.7	3.9
		....do	Oct. 13	96	6 3	18.9	4.1
6	Jan. 20	....do	Sept. 30	112	4 8	20.5	3.9
		....do	Oct. 13	99	6 3	19.2	4.1
7	Jan. 23	Ayrshire	Dec. 27	27	3 9	31.9	3.9
		....do	Sept. 27	118	5 1	22.1	3.75
		Holstein	July 17	192	5 10	24.2	3.4
8	Jan. 26	....do	May 31	240	5 9	24.9	3.3
		....do	July 20	190	4 2	23.8	3.2
9	Feb. 11	....do	Aug. 13	182	7 8	31.2	3.1
		....do	May 7	279	5 5	26.3	3.0
10	Feb. 21	Jersey	June 16	250	6 6	18.5	6.48
		....do	June 12	254	4 5	26.1	5.86

TABLE 2.—*Results of microscopical work.*

Sample No.	Size of globules.	Relative size of globules.	Percentage of globules less than one division in diameter.	Percentage of globules between one and two divisions in diameter	Percentage of globules over two divisions in diameter.
1	Small	161			
	Large	631			
2	Small	91.6			
	Large	741			
3	Small	112			
	Large	698			
4	Small	51.7			
	Large	316			
5	Small	21.7	58.3	40.4	1.3
	Large	590	15.6	38.4	46.0
6	Small	66.8	45.9	49.3	4.8
	Large	530	13.8	39.3	46.9
7	Small	12.9	73.2	26.6	0.2
	Large	703	31.6	61.1	7.3
8	Small	44.2	57.8	41.9	0.3
	Large	417	19.3	69.9	10.8
9	Small	42.3	61.2	38.5	0.3
	Large	299	14.6	80.0	5.4
10	Small	63.5	67.0	32.3	0.7
	Large	691	7.3	70.9	21.8

TABLE 3.—*Results of chemical work.*

Sample No.	Size of globules.	Specific gravity.	Melting point.	Refractive index.	Koettstorfer number.	Reichert-Meissl number.	Iodin number.	Hehner number.
1	Small			1.4609	231.2	27.29	30.91	
	Large			1.4609	230.6	27.26	30.55	
2	Small			1.4608	229.8	29.76	31.33	
	Large			1.4608	230.6	29.13	32.29	
3	Small			1.4615	234.0	25.69	32.86	
	Large			1.4613	234.6	25.49	32.83	
4	Small			1.4614	228.3	25.69	33.37	
	Large			1.4610	228.0	26.28	33.45	
5	Small[a]	0.9078	33.47	1.4565				
	Large	.9044	33.33	1.4563	232.4	23.92	29.73	87.20
6	Small[a]	.9038	33.50	1.4564	231.0		29.72	86.83
	Large	.9033	33.38	1.4562	233.3	24.15	30.20	86.83
7	Small	.9038	33.37	1.4567	229.6	25.01	30.54	86.24
	Large	.9035	33.37	1.4568	228.5	26.06	31.25	87.16
8	Small	.9053	32.37	1.4575	226.6	24.03	34.56	88.53
	Large	.9044	32.90	1.4574	226.3	24.20	34.39	88.35
9	Small	.9014	31.85	1.4575	227.6	24.98	35.42	85.73
	Large	.9014	32.43	1.4573	227.9	25.90	35.44	85.50
10	Small	.9059	32.43	1.4561	233.8	26.93	28.25	86.13
	Large	.9065	32.90	1.4560	234.9	27.63	27.62	86.83

[a] Sample too small for complete analysis.

TABLE 4.—*Color of butter and butterfat from large and small globules.*

Sample No.	Size of globules.	Butter.		Butterfat.	
		Yellow.	Red.	Yellow.	Red.
5	Small			15.5	1.5
	Large			15.5	1.5
6	Small			15.5	1.5
	Large			15.5	1.7
7	Small	0.9	0.9	27.2	1.1
	Large	.9	.9	27.2	1.6
8	Small	.8	.8	20.0	1.5
	Large	.8	.8	20.0	1.5
9	Small	.7	.8	11.0	.5
	Large	.7	.7	10.7	.3
10	Small	1.0	.8	22.0	1.2
	Large	.9	.9	21.0	1.0

CONCLUSION.

It is impossible by any known process to effect a complete separation of the large and the small fat globules in milk. The best that can be expected is to secure two creams from the milk, one of which contains a large percentage of large globules and the other a large percentage of small globules, or, in other words, one in which the relative size of the fat globules is comparatively large and another in which it is comparatively small. That such has been obtained in this investigation will be shown by Table 2. A study of Table 3 will fail to reveal any considerable differences between the fat from the large-globule cream and that from the small-globule cream. The differences in all cases are small and in most cases well within the limits of legitimate experimental error. Table 4 also shows little or no variation in color.

The investigation, of course, could be extended by introducing more animals and more samples, but the range covered by the animals selected and the number of determinations made would seem to be sufficient to warrant the conclusion that in homogeneous milk the large and small fat globules have identical chemical and physical composition.

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